

AMENDMENTS TO THE CLAIMS

The listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

Claims 1-43. (Cancelled).

44. (Currently Amended) A method for screening a library of substances compounds to detect a biologically active substance compound by detecting intracellular translocation of a subunit of a component of an intracellular pathway affecting intracellular processes, which subunit exhibits a biological activity of the component, comprising:

(a) culturing one or more cells containing a nucleotide sequence coding for a hybrid polypeptide comprising a luminophore linked to the subunit under conditions permitting expression of the nucleotide sequence,

(b) incubating the one or more cells with at least one substance compound of the library of substances compounds having unknown influences on the intracellular translocation of the subunit,

(c) screening the at least one substance of the library of compounds substances for biological function or biological effect on the subunit in the one or more cells, and

(d) measuring the light emitted from the luminophore in the incubated one or more cells and determining the result or a variation with respect to the emitted light from said luminophore, such variation being indicative of the translocation of the subunit in said one or more cells and said translocation being indicative that said at least one substance compound of the library of substances compounds to be screened is biologically active.

45. (Currently Amended) A method for screening a library of substances compounds to detect a biologically active substance compound by detecting intracellular translocation of a subunit of a component of an intracellular pathway affecting intracellular processes, which subunit exhibits a biological activity of the component, comprising:

- (a) culturing one or more cells containing a nucleotide sequence coding for a hybrid polypeptide comprising a luminophore linked to the subunit under conditions permitting expression of the nucleotide sequence,
- (b) incubating the one or more cells with at least one substance compound of the library of compounds substances having unknown influences on the intracellular translocation of the subunit,
- (c) screening the at least one substance of the library of compounds substances for biological function or biological effect on the subunit in the one or more cells, and
- (d) extracting quantitative information relating to the translocation of said subunit by recording determining a variation in spatially distributed light emitted from said luminophore, such variation being indicative of the translocation of the subunit in said one or more cells and said translocation being indicative that said substance at least one compound of the library of compounds to be screened is biologically active.

46. (Currently Amended) A method for screening a library of substances compounds to detect a biologically active substance compound by detecting intracellular translocation of a subunit of a biologically active polypeptide affecting intracellular processes, which subunit exhibits a biological activity of the polypeptide, comprising:

- (a) culturing one or more cells containing a nucleotide sequence coding for a hybrid polypeptide comprising a luminophore linked to the subunit under conditions permitting expression of the nucleotide sequence,
- (b) incubating the one or more cells with at least one substance compound of the library of substances compounds having unknown influences on the intracellular translocation of the subunit,
- (c) screening the at least one substance of the library of compounds substances for biological function or biological effect on the subunit in the one or more cells,
- (d) measuring the light emitted by the luminophore in the incubated one or more cells and determining the result or a variation with respect to the emitted light, such result or variation being indicative of the translocation of the subunit in said one or more cells and said translocation being indicative that said at least one substance compound of the library of compounds substances to be screened is biologically active, and

(e) measuring the effect of said at least one compound of library of compounds substance on the inhibition/activation of biological activity of said subunit.

47. (Previously Presented) A method according to claim 45, wherein the quantitative information relating to the translocation of the subunit is extracted from the recording or recordings according to a predetermined calibration.

48. (Currently Amended) A method according to claim 44, 45, or 46, wherein the substance compound to be screened for biological function or biological effect is a synthetic chemical compound.

49. (Currently Amended) A method according to claim 44, 45, or 46, wherein the substance compound is a substance drug whose affect on an intracellular pathway is to be determined.

50. (Previously Presented) A method according to claim 44, 45, or 46, wherein the intracellular pathway is an intracellular signaling pathway.

51. (Previously Presented) A method according to claim 44, 45, or 46, wherein the luminophore is a fluorophore.

52. (Previously Presented) A method according to claim 44, 45, or 46, wherein the luminophore is a Green Fluorescent Protein (GFP).

53. (Currently Amended) A method according to claim 52, wherein the GFP is selected from the group of GFPs having has the F64L mutation.

54. (Previously Presented) A method according to claim 52, wherein the GFP is a GFP variant selected from the group of consisting of F64L-GFP, F64L-Y66H-GFP, F64L-S65T-GFP, and EGFP.

55. (New) A method for screening a library of compounds to detect a biologically active compound that modulates intracellular translocation of at least a subunit of a component of an intracellular pathway affecting intracellular processes, the method comprising:

- (a) culturing one or more cells of at least one cell culture containing a nucleotide sequence coding for a hybrid polypeptide comprising a luminophore linked to said at least a subunit of the component under conditions that permit expression of the nucleotide sequence, said at least one cell culture being substantially devoid of any compound of the library of compounds to be screened for biological function or biological effect on said at least a subunit of the component in the one or more cells, wherein said at least a subunit of the component exhibits a biological activity of the component;
- (b) introducing at least one compound of the library of compounds into at least one of said cell cultures;
- (c) screening a plurality of compounds of the library of compounds to determine whether said at least one compound modulates the intracellular translocation of said at least a subunit of the component in the one or more cells;
- (d) measuring light emitted from the luminophore in the one or more cells to determine a first distribution of said at least a subunit of the component in the one or more cells;
- (e) measuring light emitted from the luminophore in the one or more cells to determine a second distribution of said at least a subunit of the component in the one or more cells in response to said at least one compound; and
- (f) computing a variation between the first distribution and the second distribution by processing the measured light of the first distribution and the second distribution, said variation being indicative of the translocation of said at least a subunit of the component in said one or more cells and said translocation being indicative that said at least one compound of the library of compounds is biologically active.

56. (New) A method for screening a library of compounds to detect a biologically active compound that modulates intracellular translocation of at least a subunit of a component of an intracellular pathway affecting intracellular processes, the method comprising:

- (a) culturing one or more cells of a cell culture containing a nucleotide sequence coding for a hybrid polypeptide comprising a luminophore linked to said at least a subunit of the component under conditions that permit expression of the nucleotide sequence, said cell culture

being substantially devoid of any compound of the library of compounds to be screened for biological function or biological effect on said at least a subunit of the component in the one or more cells, wherein said at least a subunit of the component exhibits a biological activity of the component;

- (b) introducing at least one compound of the library of compounds into the cell culture;
- (c) screening a plurality of compounds of the library of compounds to determine whether said at least one compound modulates the intracellular translocation of said at least a subunit of the component in the one or more cells;
- (d) measuring light emitted from the luminophore in the one or more cells at a first time point and a second time point;
- (e) processing the measured light of the first time point and the second time point; and
- (f) computing a variation in distribution of said at least a subunit of the component in the one or more cells from the first time point to the second time point, said variation in distribution of said at least a subunit of the component being in response to said at least one compound and being indicative of the translocation of said at least a subunit of the component in said one or more cells and said translocation being indicative that said at least one compound of the library of compounds is biologically active.

57. (New) A method for screening a library of compounds to detect a biologically active compound that modulates intracellular translocation of at least a subunit of a component of an intracellular pathway affecting intracellular processes, the method comprising:

- (a) culturing one or more cells of a cell culture containing a nucleotide sequence coding for a hybrid polypeptide comprising a luminophore linked to said at least a subunit of the component under conditions that permit expression of the nucleotide sequence and that are substantially devoid of any compound of the library of compounds to be screened for biological function or biological effect on said at least a subunit of the component in the one or more cells, wherein said at least a subunit of the component exhibits a biological activity of the component;
- (b) introducing at least one compound of the library of compounds into the cell culture;

- (c) screening a plurality of compounds of the library of compounds to determine whether said at least one compound modulates the intracellular translocation of said at least a subunit of the component in the one or more cells;
- (d) measuring light emitted from the luminophore in the one or more cells to determine a first distribution of said at least a subunit of the component when the cell culture is substantially devoid of any compound of the library of compounds to be screened;
- (e) measuring light emitted from the luminophore in the incubated one or more cells to determine a second distribution of said at least a subunit of the component in the one or more cells in response to said at least one compound;
- (f) processing distribution data obtained from the first distribution and the second distribution through an algorithm; and
- (g) computing a variation of said at least a subunit of the component from the first distribution to the second distribution, such variation being indicative of the translocation of said at least a subunit of the component in said one or more cells and said translocation being indicative that said at least one compound of the library of compounds is biologically active.

58. (New) A method according to claim 55, 56, or 57, further comprising processing measured light data obtained from measuring light emitted from the luminophore through an algorithm.

59. (New) A method according to claim 55, 56, or 57, wherein at least the measuring is performed with an automated system.

60. (New) A method according to claim 59, wherein at least the introducing is performed with an automated system.

61. (New) A method according to claim 55, 56, or 57, wherein at least the processing and computing is performed with a computing system.

62. (New) A method according to claim 55, 56, or 57, wherein the screening is part of a screening program to identify said at least one compound of the library of compounds to be a biological toxin or a drug target.

63. (New) A method according to claim 55, 56, or 57, further comprising fixing the one or more cells of the cell culture.

64. (New) A method according to claim 55, 56, or 57, further comprising selecting the one or more cells of the cell culture to be stable cells that are stably transformed with the nucleotide sequence coding for the hybrid polypeptide.

65. (New) A method according to claim 55, 56, or 57, wherein the component is a protein.

66. (New) A method as in claim 65, wherein said at least a subunit of the component is substantially the entire protein.

67. (New) A method as in claim 55, 56, or 57, further comprising recording a plurality of digital images of the light emitted from the luminophore.

68. (New) A method as in claim 67, further comprising implementing a digital filtering method on the plurality of digital images, said filtering method being selected from the group consisting of smoothing, sharpening, edge detection, and combinations thereof.

69. (New) A method as in claim 67, further comprising implementing a spatial frequency method on the plurality of digital images, said spatial frequency method being selected from Fourier filtering, image cross-correlation, image autocorrelation, object finding, object classification, color space manipulation for visualization, and combinations thereof.

70. (New) A method as in claim 55, 56, or 57, further comprising correlating the variation with a degree of influence of said at least one compound on the intracellular translocation of said at least a subunit of the component in the one or more cells.

71. (New) A method as in claim 44, 45, 46, 55, 56, or 57, wherein the screened compounds of the library of compounds are each screened at a known concentration.

72. (New) A method as in claim 44, 45, 46, 55, 56, or 57, wherein the screened compounds of the library of compounds are each screened at a plurality of known concentrations.